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IMMUNITY IN EXPERIMENTAL PNEUMONIA

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Clinical observations have shown that an attack of pneumonia is not, as a rule, followed by lasting immunity. Rather, the contrary is true. Osler[1] says: "No other acute disease recurs in the same individual with such frequency. Instances are on record of individuals who have had 10 or more attacks. The percentage of recurrences has been placed as high as 50. Netter gives it as 31% and he has collected the statistics of eleven observers who place the percentage at 26.8%." In the great majority of cases of pneumonia, the etiologic agent is a gram-positive coccus of one species or another. It is well known that members of this group of organisms, irrespective of their mode of entrance or the location of the lesion in the body, do not ordinarily set up a reaction on the part of the cells which results in producing a persistent marked increase in resistance. Indeed, Bunting[2] emphasizes that it is only in those diseases that show a definite reaction on the part of the lympoid tissue that we have any marked and lasting immunity. The cocci, on the contrary, quite generally stimulate the bone marrow resulting in the production of a polymorphonuclear leukocytosis.

It has been suggested by Cecil and· Blake,[3] as a result of their vaccination experiments, that it is possible that the immunity following recovery from a pneumococcus pneumonia is highly specific, and that recurring attacks may be due to one of the other fixed types not found in the initial attack or to some members of the irregular type 4.

A considerable group of workers (notably Friedberger[4] and his collaborators, and Vaughan and Wheeler[5]) hold, on the contrary, quite the opposite opinion. They maintain that the specificity of bacteria has been overemphasized and that the important points in the production of a given clinical picture are not the particular species or strain of organ-

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[1] Principles and Practice of Medicine, 1913, p. 76.

[2] Personal communication.

[3] Jour. Exper. Med., 1920, 31, p. 685.

[4] Ztschr. f. Immunitätsf., 1911, 10, p. 453.

[5] Protein Split Products, 1913.

ism but the general location of the lesions, the invasive properties of the bacteria, and special local accumulations of the organisms.

Are the frequent recurrences of pneumonia, then, due to the nature of the organism causing the disease or to some property peculiar to the lung tissue? Is it impossible for infectious processes in the lungs to stimulate the production of any considerable amount of antibodies? Since it is feasible to produce pneumonia experimentally by the use of certain gram-negative organisms which commonly induce a persistent immunity of marked degree, it has seemed possible to answer these questions. We have so little knowledge and control of the spread of respiratory infections that such answers ought to be of some value.

B. typhosus was chosen for the work planned, because it is a gram-negative organism which stimulates a high degree of immunity and is capable of exciting a pneumonic process when it is lodged in the lung. It is also markedly different from the gram-positive cocci in that it ordinarily attacks lymphoid tissue and stimulates that tissue to the production of a lymphocytosis. It was proposed to produce pneumonia by means of B. typhosus, to determine whether antibody production could thereby be stimulated and if so, to compare the agglutinin titer of the blood serum from these animals with that of animals injected intravenously.

A stock culture of B. typhosus was used. As it was avirulent for rabbits it was necessary to raise its virulence by animal passage. Attempts to do this encountered little success so long as the organisms were recovered and grown on ordinary agar. The use of Loeffler's blood serum for transplants proved more satisfactory. The same parent strain was used for the initial injection. Following the first injection, however, the organisms recovered from animals injected by each route were kept separate and reinjected always by the same path. The intravenous strain was passed through 21 rabbits, the virulence being finally raised so that one culture proved a lethal dose.

The virulence of the intratracheal strain was raised by passage through the lungs of a series of rabbits until the injection of 12 slant cultures would produce a fatal bronchopneumonia. The method of injection was as follows: After shaving the neck and disinfecting the skin, the rabbit was anesthetized and when completely relaxed was untied and the head bent back over the end of the board so as to bring the trachea up immediately under the skin. A blunt pointed needle was inserted first through the skin and then into the trachea. After the suspension was injected, the syringe was removed from the needle and the barrel filled with air which was then used to force the suspension well down into the alveoli. It was found that about 4 c c of fluid was all that could be safely injected into an ordinary sized rabbit by this method.

After injection in this manner, the rabbits crouched quietly in the cages and refused to eat. On the second day the breathing became labored and rapid and the animals were apparently ill. The symptoms reached their height on the third day. In the nonfatal cases recovery was rapid after this time. When death occurred before the third day the intestinal lesions were more marked than those in the lungs, with marked injection of the intestinal vessels and congestion of the lymphoid follicles. This distribution of the lesions, together with the fact that positive blood cultures were always obtained in these cases throughout the course of the disease, would indicate the early invasion of the blood stream in such acutely fatal cases. In animals that died later, the lesions in the lungs were more marked than those in the intestines, even when the blood cultures were positive at necropsy. The invasion of the blood stream in these cases took place only a short time before death, as the blood cultures up to that time were uniformly negative. The lungs in all of these intratracheal animals showed a well marked bronchopneumonia varying somewhat in the amount of lung tissue involved. Microscopically, the pneumonic lesions showed a considerable percentage of mononuclear cells in the exudate with relatively little fibrin and only slight evidence of hemorrhage. The interstitial tissues were heavily involved in the inflamatory process.

After the preliminary work was finished, 2 groups of rabbits were injected as indicated, one intravenously and the other intratracheally. Four cultures were used for intratracheal injections, a dose which was less than half the M L D determined in the preliminary titration. Of the 10 rabbits injected, however, 3 died on the second day, 1 on the third and 1 on the fourth, all with early pneumonic lesions and positive blood cultures. (It was thought that the excessively hot weather, during which these experiments were in progress, may have been responsible for an abrupt drop in the resistance of the animals although a sudden increase in the virulence of the bacteria may also have been a factor). The other 5 animals developed a definite pneumonia with typical symptoms and areas of consolidation but gradually recovered. Aside from the normal sample of blood taken before the injection, bleed-

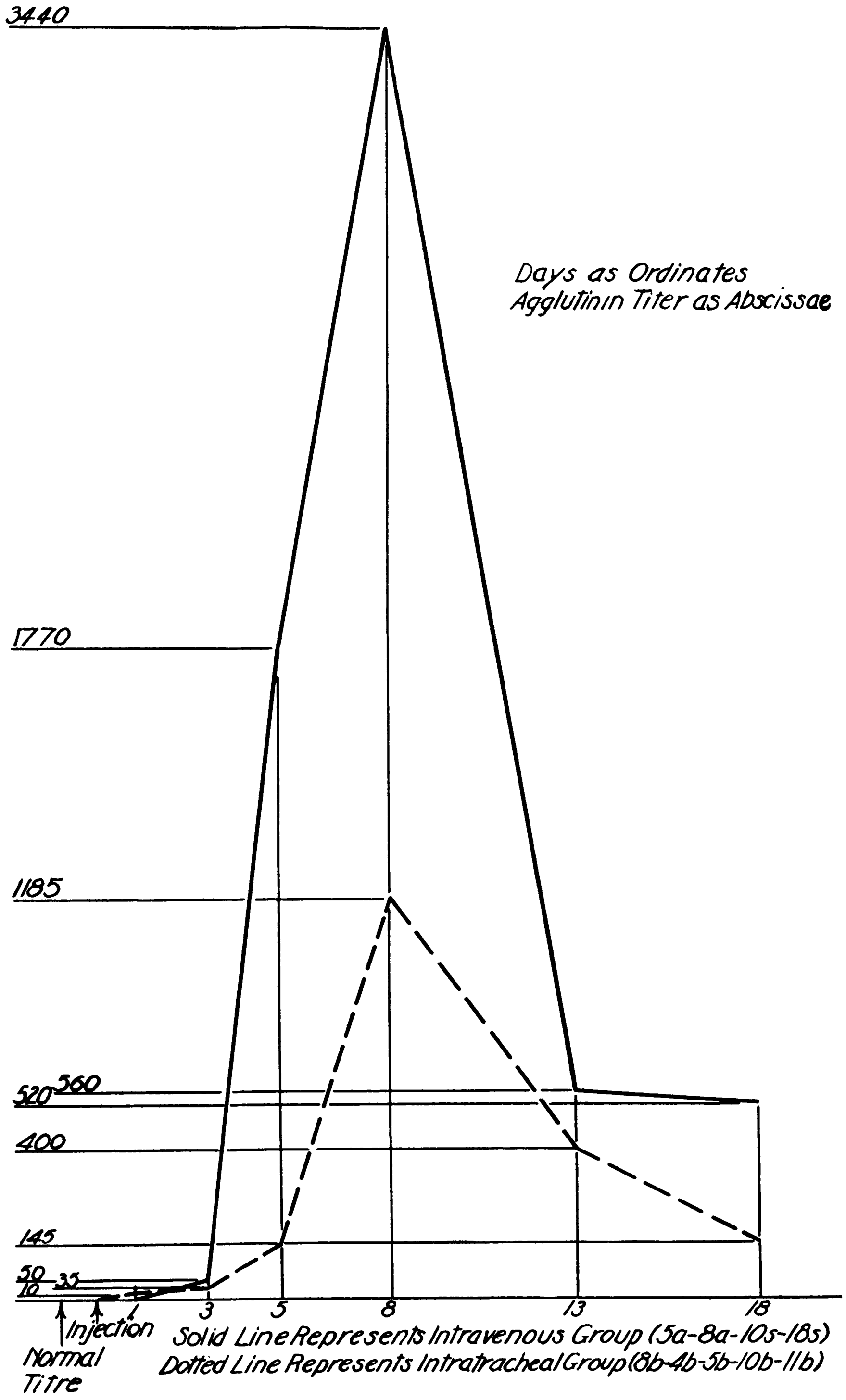

Chart 1.—Composite agglutination curves of intratracheal and intravenous groups.

TABLE 1
AGGLUTININ TITERS

Bleedings	Series A—Intravenous Injections				
	Rabbit 4a	Rabbit 5a	Rabbit 8a	Rabbit 10a	Rabbit 18a
Before injection	20—	20—	20—	20—	20—
Days after Injection:					
1	20±	20—	20±	20—	20—
3	20±	40+	80+	40+	40+
5	40+	1280+	5120+	360+	320+
8	40+	2560+	10240+	320+	640+
13	20+	1280+	640+	160+	160+
18	160+	1280+	640+	80+	80+
	Series B—Intratrachael Injections				
	Rabbit 3b	Rabbit 4b	Rabbit 5b	Rabbit 10b	Rabbit 11b
Before injection	20—	20—	20±	20—	20+
Days after Injection:					
1	20±	20±	20±	20—	40+
3	20±	80+	20—	20+	80+
5	160+	160+	40+	40+	320+
8	320+	160+	160+	160+	5120+
13	160+	320+	160+	80+	1280+
18	160+	20+	No test	80+	320+

+ indicates definite macroscopic agglutination.
± indicates an appearance slightly more granular than control; a doubtful positive.
— indicates a completely negative reaction.

TABLE 2
BLOOD COUNTS AFTER INTRAVENOUS INJECTIONS (RABBITS 4A AND 5A)

Before and Days After Injection	Red Corpuscles		Leukocytes		Polymorphonuclears		Large Lymphocytes		Small Lymphocytes		Large Mononuclears		Transitional		Eosinophils		Basophils	
	4a	5a	4a	5a	4a	5a	4a	5a	4a	5a	4a.	5a	4a	5a	4a	5a	4a	5a
Before	5,350,000	5,430,000	10,200	9,000	41	37	7	13	49	44	..	..	..	..	..	..	3	6
After																		
1 day	5,480,000	4,830,000	13,800	9,250	70	78	4	2	23	17	1	..	..	1	..	..	2	2
3 days	4,280,000	4,810,000	24,000	14,200	73	69	13	7	8	20	..	..	..	..	..	..	6	4
5 days	3,810,000	3,850.000	8,700	10,350	57	39	21	18	19	41	..	..	..	1	..	..	3	1
8 days	4,050,000	3,960,000	13,100	7,900	61	46	15	18	22	30	..	..	..	..	..	..	2	6
13 days	4,460 000	3,730,000	11,300	7,950	50	31	26	20	19	42	1	..	1	1	..	..	3	6
18 days	4,470,000	4,710,000	9,000	10,600	48	50	20	18	28	26	..	..	2	..	..	..	2	6

TABLE 3
BLOOD COUNTS AFTER INTRATRACHEAL INJECTIONS (RABBITS 10B AND 11B)

Before and Days After Injection	Red Corpuscles		Leukocytes		Polymorphonuclears		Large Lymphocytes		Small Lymphocytes		Large Mononuclears		Transitional		Eosinophils		Basophils	
	10b	11b	10b	11b	10b	11b	10b	11b	10b	11b	10b	11b	10b	11b	10b	11b	10b	11b
Before	4,690,000	5,170,000	11,950	8,100	42	22	22	19	33	57	..	..	1	..	..	..	2	2
After																		
1 day	4,880,000	5,120,000	10,300	7,650	36	68	6	12	57	20	..	..	1					
3 days	4,960,000	5,970,000	12,400	11,600	43	43	9	27	46	30	..	..	1	..	..	..	1	
5 days	5,300 000	4,210,000	8.750	10.950	57	37	22	23	20	40	..	..	1					
8 days	5,610 000	3,500 000	9,400	12,000	37	50	17	33	45	14	..	..	..	1	..	..	1	2
13 days	4,840,000	4,780,000	9,200	10.400	42	45	18	28	40	25	..	..	..	1	..	..	..	1
18 days	6,250,000	4,260,000	12,250	11,550	25	56	14	15	60	28	..	..	..	1	..	..	1	

ings were made from these animals 1, 3, 5, 8, 13 and 18 days after the injection. At each bleeding blood cultures were made in broth and on litmus lactose agar. Complete blood counts were also obtained from 2 of these rabbits at each of the bleedings.

The intravenous group, consisting of 9 animals, were injected with $\frac{1}{8}$ of a culture; the M L D previously determined was one culture. This group also showed a depression in resistance as 2 animals died within 24 hours with positive blood cultures at necropsy. Two others died of intercurrent infections on the 4th and 8th days, respectively. The 5 surviving animals were bled on the same days as the intratracheal group. Blood cultures were taken in each instance and complete blood counts in the case of 2 of these animals.

In making blood counts during the preliminary work a wide variation was observed in the number of both red and white corpuscles, depending on the manner in which the blood was obtained. If the ear vein was pricked and the pipet filled from the drop that escaped, the white count averaged several thousand and the red several million higher than in blood taken after the bleeding had continued for a few minutes. This observation was confirmed by repeated duplicate counts, and also by comparison of counts taken at the close of the bleeding with those of another worker who obtained the blood in the ordinary manner. For this work the blood for all counts was taken after the blood for serum had been collected.

All the serum obtained was preserved in sealed tubes in the refrigerator until the close of the experiment when it was titrated for agglutinins by the macroscopic method. The tubes were incubated one hour, refrigerated over night and read the next morning.

Tabulated results obtained with each animal and composite graphs of each group are given in tables 1, 2 and 3 and chart 1.

DISCUSSION

The titration of the serum for agglutinins gave some interesting facts. In both groups, intratracheal as well as intravenous, the highest antibody production noted occurred on the eighth day following the injection of B. typhosus. The intravenous group, however, attained on the average a higher level (1: 3,440) than did the intratracheal group (1:1,185). Rabbit 11 b, however, had a normal titer of 1:20 and reached a point much higher than any of the others in the intratracheal group. Rabbit 4a of the intravenous group showed a low titer—between 1: 40 and 1: 80. Its total white and polymorphonuclear leukocyte counts indicate that there was probably another infection which interfered with the usual antibody response. One rabbit, 6 b, injected intratracheally, showed no production of antibodies. These two animals therefore (4a and 6b) were not included in making the composite curves.

Blood cultures were uniformly negative in all of the nonfatal cases. This would indicate that there were few, if any, organisms circulating in the blood stream even on the first day after injection. Those injected intravenously must have been taken up by the tissues, while those injected into the lungs either remained localized or entered the blood stream so slowly as to be picked up immediately by the spleen, liver or other tissues,[6] so that they were never found in the blood cultures. Nevertheless, it is evident that the lung epithelium does not prevent the passage of B. typhosus given in large doses. The immediate deaths following intratracheal inoculation were shown by blood cultures to be due to septicemia. When death occurred after several days, however, necropsy showed that it was due to pneumonia.

The blood counts made were too few to be the basis of any definite conclusions, but are suggestive. The total white count reached its peak on the day when the clinical symptoms were most marked, and it reached a much higher level in the animals injected intravenously than in those injected by the intratracheal route. The lymphocyte count in the animals of both series showed a marked fall on the day following the injection. In the intratracheal series, this was followed by a sharp rise on the third day and a fall again on the fifth, after which, there was a gradual rise to normal on the part of one animal, and to a point above normal on the part of the other. This second animal showed a fall in polymorphonuclear leukocytes but a rise in total white cells on the eighteenth day. In the intravenous series, the lymphocyte curve reached its lowest point on the first day after injection and showed a return to normal by the fifth day. The relative proportion of large and small lymphocytes showed a marked stimulation of the lymphoid tissues following the initial destruction and injury. At the time of greatest antibody content, the blood counts had returned practically to normal and the animals were apparently well.

Although it is obvious that a larger series of animals would give a more adequate basis for conclusions, it is evident that considerable antibody production can be induced by B. typhosus when that organism is used to produce a pulmonary lesion in rabbits. As we should expect, the absorption of the antigen is less and the antibody response is less than when, by means of intravenous injection, there is widely scattered opportunity for absorption of the antigen. The relatively greater resistance of the body when subjected to attack through the lung tissue has again been demonstrated in that by the intratracheal route the animals withstood many fatal doses as measured by intravenous tolerance. Possibly this is due partly to the fact that most of the organisms do not pass through the alveolar wall into the blood stream, and also to the failure of the alveolar epithelium to anchor physiologically much of the antigen.

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[6] Bull, Jour. Exper. Med., 1915, 22, p. 475. Drinker: Ibid., 1921, 33, p. 77.

A study of the bone marrow in connection with the blood counts should offer further information of interest, and a comparison of antibodies resulting from localized lesions in other parts of the body should be undertaken.

CONCLUSIONS

The injection of B. typhosus by the intratracheal route causes bronchopneumonia, and coincidentally with recovery specific agglutinins are found in the blood.

The agglutinin titer in the group animals with pneumonia does not reach as high a level as in the group of intravenously treated animals but the peak of the curve is reached on the same day, the eighth following the injection of the bacteria.

The failure ot attacks of pneumonia to produce any considerable immunity is probably due, therefore, to the nature of the organism causing the disease rather than to any peculiar properties inherent in the lung tissue.